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Micrometric Numeration of Blood-Corpuscles.

by

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On the Micrometric Numeration *of the* Blood-corpuscles *and the* Estimation *of their* Hæmoglobin. By Mrs. Ernest Hart.

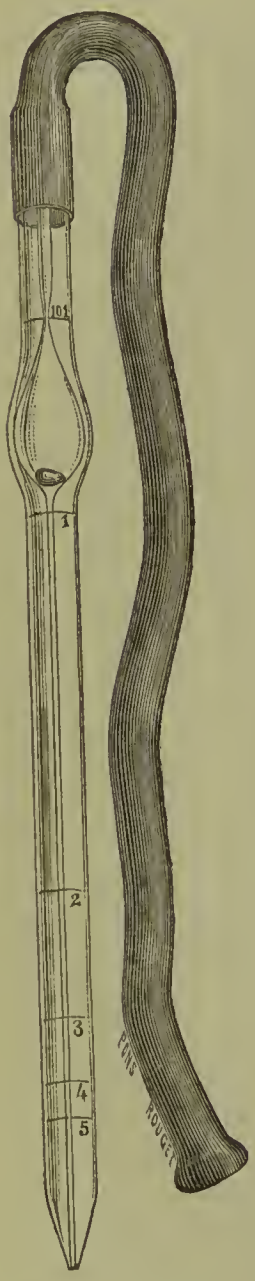
The micrometric numeration of the blood-corpuscles and the estimation of hæmoglobin are operations which, though of comparatively recent introduction, have rapidly passed out of the sphere of laboratory experiment into practical use as exact methods of physiological and clinical investigation. Those who have worked at this subject cannot, however, have proceeded far without discovering that the methods and instruments hitherto in use are inconveniently imperfect and vitiated by numerous sources of error. Some recent improvements by M. Malassez, assistant in the Laboratory of Histology in the Collége de France, appear to me to have done much to remove these disadvantages.

Before proceeding, however, to describe the new Corpuscle-Counter which M. Malassez has just introduced, it may be well to say a few words on the methods and instruments usually employed for the numeration of the corpuscles. The three which have been hitherto in general use are those known as the instruments of Malassez, Hayem, and Gowers. In the method first invented by Malassez (the *Compte-Globules Capillaire*) 100 parts of a 5 per cent. solution of sulphate of soda are mixed in a special instrument called the *Melangeur Potain* (Fig. 1) with one part of blood. This solution is then drawn into an extremely fine capillary tube. The calibre of this tube is known; hence the volume of the fluid which the tube contains in a given length, say in 500, 400, or 300 micro-millimeters is also known.[1] This volume is some fraction of a cubic millimeter. It follows that the volume multiplied by the denominator of that fraction will equal a cubic millimeter. The multiplier is written on a glass plate, on which the capillary tube is mounted. Before using the instrument the eye-piece of the microscope must be exchanged for an eye-piece containing a micrometer divided into a number of square millimeters. Then by means of a stage micrometer, the microscope must be graduated, so that ten of the square millimeters of the eye-piece correspond exactly to the arbitrary length (500μ, 400μ, or 300μ) fixed upon. A mark being then put on the tube of the microscope, this magnifying power—the lens being always the same—can be easily found again. The process and calculation are then

[1] This unit, the thousandth of a millimeter, is expressed by the Greek μ.

very simple. The solution of blood, after being well shaken in the Mixer, is drawn up into the tube by capillary attraction, and the number of corpuscles contained in the given length of the tube is counted. This is rendered extremely easy by the aid of the small squares of the ocular micrometer. The number obtained is multiplied by the denominator written, as already mentioned, on the glass plate, and the product multiplied by 100 or 200, according to the strength of the solution used, gives the number of the corpuscles per cubic millimeter. Thus, supposing that, in a length of 500μ, the volume $=\frac{1}{150}$ of a cubic millimeter, and that a 1 per cent. solution occupying this space contains 300 corpuscles, then

Fig. 1.



$$300 \times 150 \times 100 = 4{,}500{,}000$$

the number of corpuscles per cubic millimeter of blood. The calculation, however, is not so simple as this in practice, since the multiplier, of course, will seldom be a round number.

M. Ranvier, in his 'Traité Technique d'Histologie,' says that this method, considering the short time it takes, gives, of all those hitherto known the best results. It is certainly very accurate, but it has two great disadvantages. In the first place, owing to the necessity of undertaking the somewhat difficult task of graduating the microscope, the same *Compte-Globules* must always be used with the same microscope and the same lens; hence its use clinically is obviously very much curtailed. Secondly, the extreme delicacy of the instrument is a serious drawback. Though a person with ordinary manipulative skill may learn to use this instrument, it requires more than ordinary care to keep the minute capillary tube absolutely clean, and this is positively necessary, since a minute particle of dirt, or a few dried corpuscles left in the tube, will vitiate the accuracy of the results. My own experience is that, though I am able to make correct observations with this

instrument in the pure air of Paris, I am unable in London, where nothing is clean, not even distilled water, to keep the tube quite free from dirt.

Hayem's method differs altogether from that of Malassez. The unit is arrived at by means of a cell $\frac{1}{5}$ mm. deep, of which an area of $\frac{1}{20}$ of a square mm. is marked off. This gives us, therefore, $\frac{1}{5} \times \frac{1}{20} = \frac{1}{100}$ of a mm^3. The area of $\frac{1}{20}$ square mm. (which is, of course, $\frac{1}{20}$ of 1,000,000 square μ) is obtained by using an ocular micrometer, on which is drawn an oblong, 5 mm. long by 4 mm. wide, and divided into 20 squares. By means of a stage micrometer the microscope is graduated so that the 5 mm. exactly correspond to an objective length of 250μ. The mixture of blood and the preserving fluid is made at a strength of 8 per 1000. The method of mixing is that invented by Vierordt. A pipette, holding 8 cubic mm., is used to measure the blood; another, of a calibre of 992 cubic mm., to measure the preserving solution. The two fluids are mixed in an open glass vessel by means of a glass rod. The same rod is also used to deposit the drop on the slide. The cover-glass is kept *in situ* by the capillary attraction existing between two moist glass surfaces, a drop of water or saliva being placed at the edge of the cover-glass, and allowed to run under it. The chief objections to this instrument are the uncertain depth of the cell, the clumsy method of mixing, the possible elevation of the cover-glass by allowing too much water to run under, it and also the same objection made to Malassez's capillary *Compte-Globules* just considered, namely, that, owing to the necessity of graduating the microscope, it is of limited use as a clinical instrument.

In Gowers' Hæmacytometer,[1] which is a modification of Hayem's, a very decided improvement is made. In the depth of the cell and in the old-fashioned mode of mixing, it is identical with that of Hayem; the solution of blood used being, however, at 5 per 1000 instead of at 8 per 1000. The improvement consists in measuring the area and drawing the squares in which the corpuscles are to be counted upon the floor of the cell itself. Squares, with sides $\frac{1}{10}$ of a mm. long, are drawn on the floor of the cell. The area of each, therefore, is $\frac{1}{100}$ of a sq. mm. The cell having a depth of $\frac{1}{5}$ mm., and any 10 squares an area of $\frac{1}{10}$ of a sq. mm., the cubic contents of any ten squares taken within the cell will be—

[1] "On the Numeration of the Blood-corpuscles," by Dr. Gowers, 'Lancet,' Dec., 1877.

$$\tfrac{1}{10} \times \tfrac{1}{5} = \tfrac{1}{50}\ \text{mm}^3.$$

The number of corpuscles observed in ten squares, therefore, multiplied by fifty will give the number in a cubic millimeter of the solution; and that multiplied by $\frac{1}{2000}$ will give the result for a cubic millimeter of blood.

For example, if the number of red corpuscles counted on ten squares is 500, the calculation is simply

$$500 \times 50 \times \frac{1000}{5} = 500 \times 10{,}000 = 5{,}000{,}000 \text{ per mm.}^3,$$

or, in other words, there is nothing to be done except to add 0000 to the number found by counting. No graduation of the microscope is required, so that the instrument can be used anywhere and with any lens. This renders it more convenient as a clinical instrument, and it is therefore that which is in general use in the English hospitals. It gives approximately accurate clinical results. I must, however, point out that it is liable to four serious sources of error, which destroy the value of observations made with it from an absolute and scientific point of view. These sources of error are—the uncertain depth of the cell; the inequality of the surface of the cover-glass; the method of placing the cover-glass on the drop; and the means used to make the mixture and to place the drop in the cell. Since a paper by two American physicians was published,[1] showing how careful observations may be vitiated by the variation in the depth of the cell in different instruments, the error in the depth has been written on the slide. In the Hæmacytometer which I habitually use the cell has a depth of 190μ, instead of 200μ. This error necessitates a troublesome correction in each calculation. The correction is made by multiplying the number of corpuscles obtained by 20 and dividing by 19; for let a equal the number of corpuscles in a mm.^{2} multiplied by the actual depth of the cell,

$$190\mu : a :: 200\mu : x.$$

This method of correction which is that recommended is, however, irksome when a great number of observations have to be made. I wish now to suggest that it may be altogether avoided by directing the instrument maker to graduate the pipette or mixer, whichever may be used, not, as at present, on the assumption that the depth of the cell accurately measures 200μ, and therefore that a solution of 5

[1] "Blood-Cell Counting: a Series of Observations with the Hématimètre of M.M. Hayem and Nachet, and the Hæmacytometer of Dr. Gowers." By Drs. Henry and Naucrede.—'Boston Med. and Surg. Journ.,' April, 1879.

per 1000 should be used to ensure correct results, but so to graduate it as to make a solution of such a strength that, having previously ascertained the actual depth of the cell an area of $\frac{1}{10}$ mm.^{2} multiplied by this depth shall give $\frac{1}{5}\frac{1}{0}$ mm.^{3} In this way the necessity for arithmetical correction of each observation is avoided, the special adjustment of the pipette affording a correction which applies to all observations made with the instrument. Thus, taking my own Hæmacytometer as an example, if, instead of using a 5 per 1000 solution, a 5 per 950 solution were used, *i. e.* 5 parts of blood to 945 of the diluting fluid, the result would be absolutely the same as if the depth of the cell were correct, or as if the error were corrected by calculation. Thus supposing 500 corpuscles to be contained in ten of the squares,

$$500 \times 10 \times \frac{1000}{190} \times \frac{950}{5} = 5{,}000{,}000.$$

This device will work equally well whatever the error in the cell may be, if the following rule be adhered to:— Multiply the actual number of μ in the depth of the cell by 5 and take the product as the number of parts of the solution of blood and diluting fluid to be used, the number of the parts of blood remaining constant at five—

$$190\mu \times 5 = 950$$

or, still more generally, the number of parts of blood being fixed, and the actual depth of the cell in μ being known, the product of these two numbers, minus the number of the parts of blood, will give the necessary number of parts of diluting fluid required.

With the pipette or mixer graduated according to these rules, it will only be necessary to add 0000 to the number of corpuscles counted in ten squares.

I commend this suggestion to the notice of all who are using Gowers' instrument, as its adoption will greatly facilitate the attainment of correct results.

Secondly, as to the error caused by the inequality of the surface of the cover-glass. Any ordinary cover-glass is used to flatten the drop to an uniform height. Now, as every histologist knows, cover-glasses are rarely of an uniform flatness; they are generally either slightly convex or concave, hence the layer of fluid is likely to be thicker in some places than in others, and consequently a count made in one part of the cell may give very different results from one made in another. To remedy this defect in my instrument, I have had ground a perfectly flat cover-glass.

Thirdly, the mode of placing the cover-glass on the cell

is faulty; whether it is dropped on horizontally or laid on gently at an inclined plane, the uniform diffusion of the corpuscles through the fluid is disturbed.

Fourthly, in the method of mixing and placing the drop on the cell, errors are caused by the white corpuscles adhering to the sides of the vessels used for mixing, and by evaporation from the little open cup in which the solution is kept. Further, in placing the drop on the slide, unless the manœuvre is very quickly executed, the red corpuscles gravitate to the bottom of the drop, and are thus deposited and form a thicker collection in the centre of the drop than at the periphery. The white corpuscles also, by adhering to the glass rod, introduce a source of error in estimating the right proportion between white and red corpuscles.

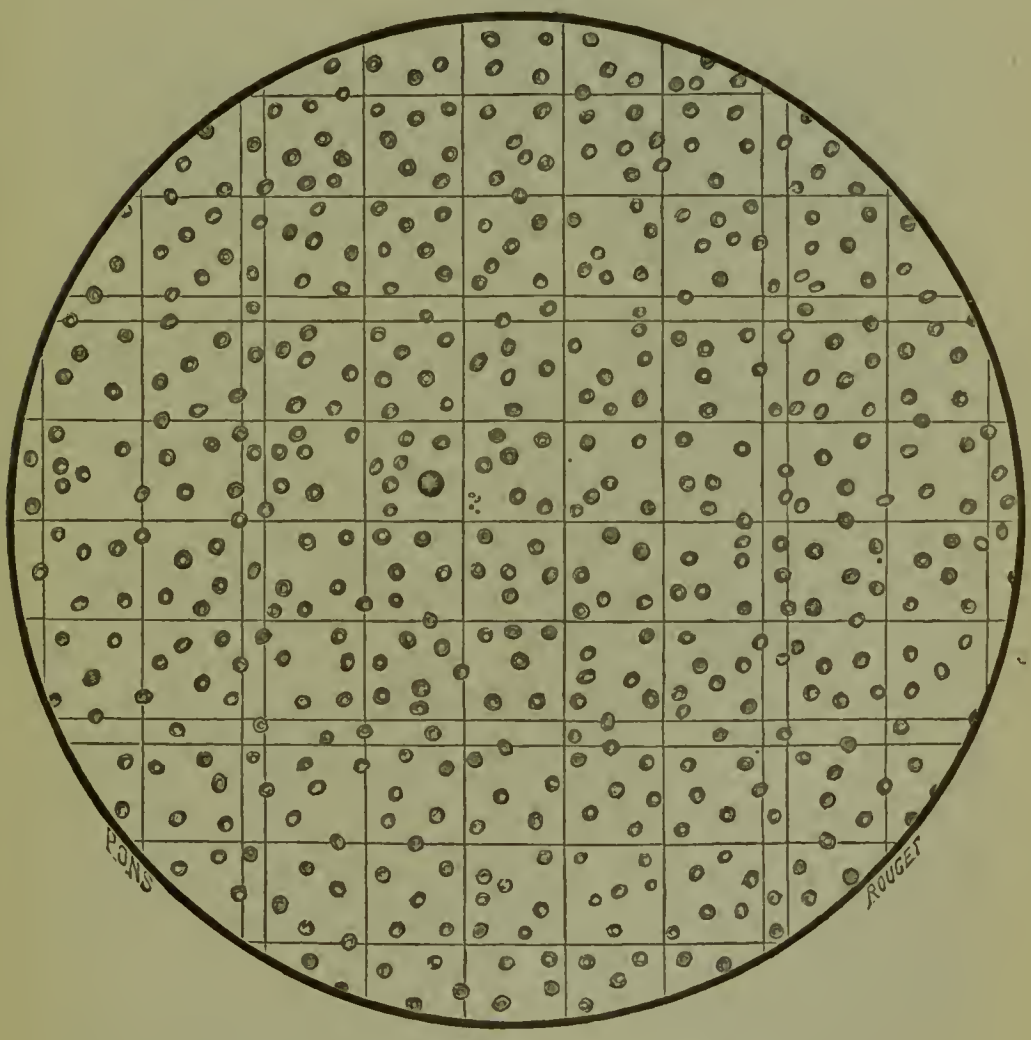
It is, I think, to be regretted that, in introducing this really useful clinical instrument, Dr. Gowers should have adopted the old, clumsy, and discarded method of making the solution, instead of using Potain's Mixer, the use and value of which were already known. By this mixer a solution of blood at 100, 200, 300, 400, or 500, as desired, is made in a *closed* vessel, evaporation thus being prevented; the drop is deposited on the slide whilst the corpuscles are in rapid motion and before they have had time to gravitate to the bottom of the drop. For the last eighteen months I have, when using Gowers' Hæmacytometer, substituted Potain's Mixer in the place of the apparatus provided, and with the result of obtaining much more uniform counts in different parts of the cell, whereas previously the want of uniformity was often very marked.

By the means I have indicated, namely, by correcting the error in the depth of the cell, by substituting a perfectly flat cover-glass for one that may or may not be flat, and by using Potain's Mixer for making the solution, a useful and nearly accurate clinical instrument can be made of Gowers' Hæmacytometer. As it is at present arranged, the results obtained by it are often misleading, unless the mean of a great number of counts be taken. Single observations are likely to lead to the most fallacious conclusions, and are not at all trustworthy, whether for scientific or clinical data.

In Malassez's new *Compte-Globules* he has adopted the great improvement introduced by Gowers, of drawing the squares on the surface of the slide. He has moreover succeeded, by many ingenious contrivances in carefully avoiding all the sources of error in Hayem's and Gowers' instruments above enumerated, to several of which I had occasion to call his attention. This new MICROMETRIC GRA-

DUATED CORPUSCLE-COUNTER with WET CHAMBER (*Compte-Globules à chambre humide graduée micrometrique*[1]) consists of a thick nickel slide, in the centre of which is a circular groove enclosing a glass cylinder about a centimeter in diameter. Outside this groove are three pointed metal screws, equidistant from each other. The elevation of these points above the surface of the metal slide is exactly $\frac{1}{5}$ mm. In the centre of the glass surface, limited by the groove, are drawn the squares, in which the corpuscles are counted. These have a side of $\frac{1}{20}$ mm., and they are arranged in groups of 20, each group having a length of $\frac{5}{20} = \frac{1}{4}$ mm., and a width of $\frac{4}{20} = \frac{1}{5}$ mm., and an area, therefore, of $\frac{1}{5} \times \frac{1}{4} = \frac{1}{20}$ square

FIG. 2.

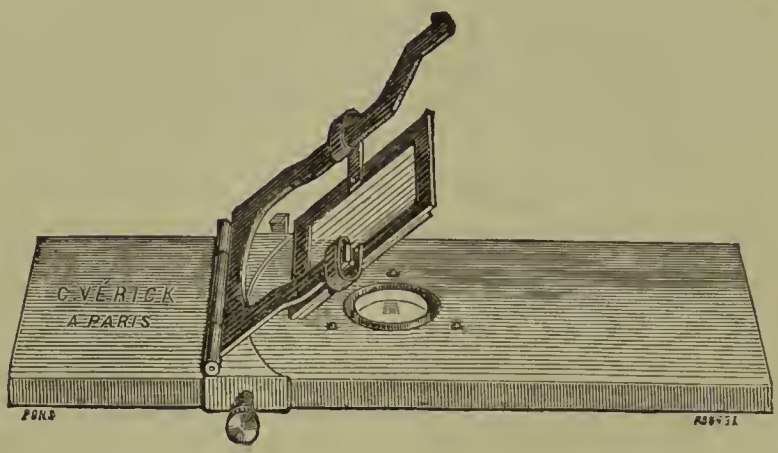


mm. Each group of 20 squares is separated from adjoining groups by a double line (Fig. 2). The peripheral

[1] "Sur les Perfectionnements les plus récents apportés aux Méthodes et aux Appareils de Numération des Globules Sanguins, et sur un nouveau Compte-Globules," par L. Malassez, 'Arch. de Phy.'

parts of the ruled space are simply divided into rectangles, $\frac{1}{4}$ mm. long and $\frac{1}{5}$ mm. wide. The cover-glass, which is ground accurately flat, is attached, by moistening the edges slightly with saliva, to a frame fixed to the sides of the slide. By an ingenious and delicate rack movement of this frame the cover glass is lowered without delay, and *in a horizontal position* down upon the drop. The slide carrying the frame is represented in Fig. 3.

Fig. 3.



To make a numeration, the solution is made in Potain's Mixer at the strength of 1 per 100, 200, 300, 400, or 500, as desired; and, whilst being rapidly agitated, a drop is placed in the centre of the ruled space, and the cover-glass, having been previously attached to the frame, is lowered and clipped, so as to rest firmly on the points of the screws. To prevent evaporation, if desired to keep the preparation any length of time, a drop of water should be placed at the edge of the cover-glass, and allowed to run under and fill the vacant space between its edge and the groove. The *red* corpuscles that are lying within a group of 20 squares are then counted. These 20 squares, it will be remembered, have an area of $\frac{1}{20}$ mm.^{2}, and the depth of the fluid being $\frac{1}{5}$ mm., the quantity of the solution under review will be $\frac{1}{20} \times \frac{1}{5} = \frac{1}{100}$ of a mm.^{3} The number of corpuscles seen, therefore, has to be multiplied by 100, and then again by the number representing the strength of the solution, and the product will be as before the number of corpuscles in a cubic millimeter of blood.

Thus, for example, let the solution be 1 per 200, and let 250 corpuscles be found on an area of $\frac{1}{20}$ mm.^{2}; then—

$$250 \times 100 \times 200 = 5{,}000{,}000.$$

Thus, to the number of corpuscles counted, if the solution

be 1 per cent., it is only necessary to add 0000, but if the strength of the solution be less it is necessary to multiply the number of corpuscles by the figure representing the dilution before adding 0000. To correctly estimate the number of *white* corpuscles per cubic millimeter a much larger area must be taken, and for this purpose the rectangles of $\frac{1}{20}$ square mm. have been drawn on the slide. The number of white corpuscles found in ten of these large rectangles must be counted. If in a 1 per cent. solution the number of white corpuscles in ten of these large rectangles is found to be thirty, then we know, as above shown, that the volume of the solution counted is—

$$10 \times \tfrac{1}{20} \times \tfrac{1}{5} \text{ or } 10 \times \tfrac{1}{100} = \tfrac{1}{10} \text{ mm.}^3$$

The number counted, therefore, multiplied by 10 and then by 100, will give at once the number of white corpuscles in a cubic mm. of blood; or, in other words, it is only necessary, for a one per cent. solution, to count and add 000. For example:

$$30 \times 10 \times 100 = 30{,}000.$$

This method of estimating the number of white corpucles will be felt by every worker at this subject to be a great gain, for on this point none of the previous instruments gave any but the roughest approximate results, likely to give rise to the most delusive conclusions. To sum up, the advantages of this new *Compte-Globules* over that first introduced by M. Malassez are that it can be used clinically with any microscope, that no particular skill is required to use it, and only ordinary care to keep it clean and in order. Over other clinical Corpuscle-Counters it possesses the merits—of making the layer of fluid accurately $\frac{1}{5}$ mm. in depth, so that there are no corrections to make; of having the squares ruled to the smallest size yet found possible, so that the numeration is exceedingly easy and not fatiguing to the eyesight; of making an exact computation of the number of white corpuscles per cubic mm.; and, lastly, by means of the rack movement of the carrier of the cover-glass, and by the use of the Melangeur Potain, of preserving the homogeneity of the drop when placed on the slide and flattened to the depth of $\frac{1}{5}$ mm.

The counting of blood-corpuscles is now so common and frequent an operation in clinical medicine, and its value in assisting diagnosis and treatment is so well recognised, that I feel sure that insistence on the minute details and scrupu-

lous care necessary to ensure correct and reliable results will not be thought trivial.

Corpuscle counting is, however, only one stage in the optical investigation into the state of the blood. To arrive at an opinion on which diagnosis and treatment should be based, it is necessary to estimate the amount of hæmoglobin as well. In an elaborate paper[1] of Malassez (of which I published an abstract in the 'London Medical Record' of 1879), all the various methods employed for estimating hæmoglobin are described at length. In nearly all of these an arbitrary standard of colour is taken as normal, and the blood to be examined is compared with it. In Malassez's Hæmochromometer there is no arbitrary standard; each degree of the coloured standard solution to which the blood is compared corresponds to a blood containing a certain estimated amount of hæmoglobin per cubic mm., and having the power of absorbing a certain known amount of oxygen.

These figures have all been ascertained by a prolonged series of experiments; here therefore, there is no guessing that the amount of hæmoglobin may be above or below the normal, for we are able to ascertain the actual amount of hæmoglobin in a cubic mm. of blood, and also the respiratory power of the same unit. But M. Malassez points out that it is not only necessary to ascertain the amount of hæmoglobin per cubic mm., but that we should learn in what state of division it exists, namely, what is the amount contained in each corpuscle. Welcker considers that there is always a constant relation physiologically between the richness of the blood in corpuscles and in hæmoglobin; Hayem and Johann Duncan have, however, discovered that, pathologically, particularly in anæmia and chlorosis, the relations are disturbed, the number of corpuscles often resting normal, the hæmoglobin being less than normal. The way of arriving at the amount of hæmoglobin per corpuscle is, by M. Malassez's method, extremely simple. The number of corpuscles in a cubic mm. of blood is first counted, and by the hæmochromometer the amount of hæmoglobin per cubic mm. is estimated. The latter figure divided by the former gives the amount of hæmoglobin per corpuscle. Thus, a blood containing 5,000,000 corpuscles per cubic mm., and 0·125 mlgr. of hæmoglobin per mm.^{3} gives $\frac{0{\cdot}125}{5,000,000} = {\cdot}000,000,025$ mlgr., *i. e.* $\frac{25}{1,000,000,000}$ of a $\frac{1}{1,000,000}$ of a gramme, or, as it is commonly written,

[1] "Sur les diverses Méthodes de Dosage de L'Hémoglobine et sur un nouveau Colorimètre," par L. Malassez, 'Arch. de Phy.,' 1877.

25 $\mu\mu$ gr. The result in terms of $\mu\mu$ gr., however, may be found in a moment by simply dividing 125 by 5 = 25, and disregarding all ciphers.

In an extremely interesting research,[1] M. Malassez found that, pathologically, the estimation of the hæmoglobin per corpuscle gave very significant indications. In a case, which he quotes, of chlorosis which improved under treatment, the actual number of corpuscles per cubic mm. diminished, the amonnt of hæmoglobin per corpuscle, almost doubling, however, in the same time; mere corpuscle counting here would have given an erroneous inference. In a series of experiments on fowls kept first at liberty in the open air, and then in unhealthy conditions in a courtyard, it was found that though the corpuscles did not notably diminish in number, the hæmoglobin per corpuscle fell from 48 $\mu\mu$ gr. to 33 $\mu\mu$ gr. On examining a great number of animals he found that the lower in the scale one descends the larger the amount of hæmoglobin per corpuscle, so that it might be too hastily assumed that the blood of the lower animals was richer in hæmoglobin than that of the higher. At one end of the scale stands man with a mean normal of 30 $\mu\mu$ gr., and at the other the Proteus with 1066·6 $\mu\mu$ gr. But the corpuscle of the Proteus is 127 times the volume of that of the human subject. The true ratio between them can only be ascertained by knowing the amount of hæmoglobin contained in an unit of corpuscular substance. The unit taken is μ^3. To obtain this, the volume of the corpuscle must be known. Welcker, by an elaborate process, ascertained the mean volume of the corpuscles of a few animals as standards of comparison. These measurements being accepted as accurate, the amount of hæmoglobin per corpuscle is divided by the mean volume of the corpuscles, and the product is the amount of hæmoglobin per μ^3 of corpuscular substance.

From the following table it will be seen that though the quantity of hæmoglobin per corpuscle may increase from the higher to the lower animals, the true ratio of comparison is the unit of corpuscular richness in hæmoglobin, and that this, on the contrary, rises in passing from the lower to the higher animals:

Welcker's ingenious method of ascertaining the value of

[1] "Sur la Richesse en Hémoglobine des Globules rouges du Sang," par L. Malassez, 'Arch. de Phy.'

	Quantity of hæmoglobin per corpuscle.	Volume of the corpuscle.	Quantity of hæmoglobin per μ cube of corpuscular substance.
	$\mu\mu$ gr.	μ cube.	$\mu\mu$ gr.
Man . .	30	72	0·4166
Pigeon .	52	125	0·4160
Lizard .	70	201	0·3483
Russian frog .	216	629	0·3432
Proteus . . .	1066	9200	0·1159

the volume of the corpuscles in μ^3 is, however, quite out of the question in clinical work, and as no simpler method has at present been devised, we must it appears to me, be at present content to ascertain the mean area of the corpuscles in μ^2, and to take as our unit of corpuscular substance μ^2 multiplied by the unknown thickness of the corpuscle, on the assumption that this is uniform throughout, and always the same. Actually we know this not to be the case, as normal corpuscles are biconcave and not flat, and in pathological conditions they vary in form, and possibly in thickness. However, let the constant representing the supposed thickness (or more accurately the factor by which we should multiply the diameter to obtain the volume) be called τ. Our arbitrary unit of corpuscular substance will, therefore, be $\tau\mu^2$. The results in the way of comparison will only be liable to error in so far as the corpuscles vary in thickness. As, however, this variation is immeasurable by our present instruments, it may be taken that the unit $\tau\mu^2$ will give for all practical purposes a sufficient approximation to the truth.

To obtain the mean area of the corpuscles in any given specimen of human blood, the mean diameter of the corpuscles must first be ascertained. A simple method of obtaining this is to graduate the microscope so that an image thrown by the camera lucida at a certain fixed distance magnifies exactly 1000 diameters. The corpuscles having been rapidly fixed and dried by exposing them to the action of heat, or better still, to the vapour of osmic acid, their image is thrown by the camera lucida on to white paper, care being taken to correct the errors of refraction.[1] The outlines of the corpuscles are then traced in pencil, and their diameters

[1] 'Note sur la Mesure des Grossissements Microscopiques,' par L Malassez. 'Correction des Déformations produites par les Chambres Claires de Milne-Edwards et de Nachet,' par L. Malassez.

measured by a millimeter rule. The resulting numbers give the diameters of the corpuscles in micro-millimeters. Of course the mean of a great number of measurements must be taken. I generally take the mean of fifty measurements. The area in μ^2 is obtained by the well known formula of πr^2.

To take an example:

Let $7{\cdot}60\mu$ = the diameter of a normal corpuscle;

and $3{\cdot}8\mu$ = the radius: then

$$\pi r^2 = \pi . 14{\cdot}44\mu^2 = 45{\cdot}36\mu^2.$$

$45.36\mu^2$ is therefore the area of a normal human corpuscle. To obtain the unit of corpuscular richness, *i. e.* the amount of hæmoglobin in a volume of $\tau\mu^2$, the amount of hæmoglobin per corpuscle must be divided by the area.

In the following table, which I have prepared, these calculations, and others to which reference has been made above, have been worked out. The number of corpuscles in a cubic millimeter is taken as invariable in the examples of normal and anæmic blood (this is not infrequently the case); all the other figures vary, however, from the normal in different degrees, the unit of corpuscular richness being finally the only figure that gives the exact ratio between the normal and pathological states.

	No. of corpuscles per mm.³ of blood.	Hæmoglobin per mm.³ of blood.	Hæmoglobin per corpuscle.	No. of corpuscles per mil. gr. of hæmoglobin.	Diameter of corpuscle.	Area of corpuscle.	Hæmoglobin per unit $\tau\mu_2$ of corpuscular substance.
		m. gr.	$\mu\mu$ gr.		μ.	$\mu.^2$	$\mu\mu$ gr.
The corpuscles in a normal state of health .	4,500,000	0·134	29·77	33,580,000	7·60	45·36	0·66
Slight anæmia, with diminution of the size of the corpuscle .	4,500,000	0·082	18·66	54,878,048	6·50	33·18	0·56
Marked anæmia, with increase of the size of the corpuscle . .	4,500,000	0·062	13·77	72,580,645	8·20	52·81	0·26

Pathologically these exact or minute analyses are most interesting, though their outcome clinically and therapeuti-

cally is yet obscure. But we may hope that a minute study of the state of the blood in the cachexia of cancer and syphilis, in pernicious and simple anæmia, in chlorosis and leucocythæmia and in other wasting diseases, may lead to an exact knowledge of the pathological changes, revealing the causes at work and that this knowledge may form a rational basis of treatment.